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(54) **Immunoregulatory diketopiperazine compounds.**

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Field of the Invention

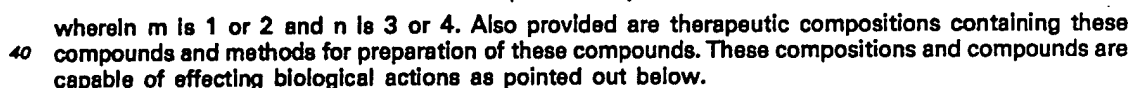
Description of the Prior Art

Reference is made to this patent and patent application for a detailed discussion of other prior art and the biological processes involved in the present invention. In US—A—3,763,091 a series of 15 certain diketopiperazines are disclosed which are starting products for the preparation of polymers. No immunological or biological properties are described in this document.

The present invention provides diketopiperazines (cyclic dipeptides) which also have potent ubiquitin-like activity. Since ubiquitin itself and the peptides disclosed in the referenced patent application are all linear, it is most surprising that the subject diketopiperazines, which are cyclic, possess this same utility.

It is therefore one object of this invention to provide new immunoregulatory diketopiperazines which are highly useful in the immune system of humans and animals. It is a further object to provide 25 pharmaceutical compositions and methods for preparing them.

Other objects and advantages of the invention will become apparent as the description proceeds. In satisfaction of the foregoing objects and advantages, there is provided by this invention the novel 2,5-diketopiperazine compounds of formula:



It will be apparent from an examination of the above formula that the subject compounds contain two asymmetric centers and can therefore exist in a variety of optically active forms. That is, the optically active centers may be both dextro (D), both levo (L), or one may be dextro and the other levo. All of these possible combinations of optical isomers are intended to be included within the subject invention.

The subject substituted diketopiperazines combine simplicity of structure, ease of manufacture, and high immunoregulatory potency, and thus provide significant advantages.

As indicated above, this invention is concerned with new diketopiperazine compounds, therapeutic compositions containing these compounds, and methods for manufacture thereof.

In its broadest scope, the present invention provides 2,5-diketopiperazine compounds having the following formula:



and optical isomers thereof, wherein m is 1 or 2 and n is 3 or 4.

Also included within the scope of the invention are the pharmaceutically acceptable salts of these compounds. As acids which are able to form salts with the compounds, there may be mentioned inorganic acids such as hydrochloric acid, hydrobromic acid, perchloric acid, nitric acid, thiocyanic acid, sulfuric acid, phosphoric acid, *tc.*, and organic acids such as formic acid, acetic acid, propionic acid, glycolic acid, lactic acid, pyruvic acid, oxalic acid, malonic acid, succinic acid, maleic acid, fumaric acid, anthranilic acid, cinnamic acid, naphthalenesulfonic acid, and sulfanilic acid, for example.

It will be understood that the subject diketopiperazines may be viewed as cyclic dipeptides which may comprise as one of the amino acid constituents either D or L-glutamine or D- or L-asparagine; and as the other amino acid constituent either D- or L-lysine or D- or L-ornithine. The following are given as examples of cyclic dipeptides included within the generic formula given above: cyclo-(GLN-LYS), cyclo-(GLN-ORN), cyclo-(ASN-LYS), and cyclo-(ASN-ORN); cyclo-(D-GLN-D-LYS), cyclo-(D-GLN-D-ORN), cyclo-(D-ASN-D-LYS), and cyclo-(D-ASN-D-ORN); and cyclo-(D-GLN-LYS), cyclo-(GLN-D-LYS), cyclo-(ASN-D-LYS), cyclo-(GLN-D-ORN), cyclo-(D-ASN-ORN), and so forth.

In the above structures, the amino acid components of the cyclic dipeptides are identified by abbreviations for convenience. These abbreviations are as follows:

	Amino Acid	Abbreviation
20	L-glutamine	GLN
	L-asparagine	ASN
25	L-lysine	LYS
	L-ornithine	ORN
30	D-glutamine	D-GLN
	D-asparagine	D-ASN
	D-lysine	D-LYS
35	D-ornithine	D-ORN

The subject compounds may be prepared by various techniques following principles generally known in the art. Synthesis of cyclic peptides is described, for example, in M. Bodanszky, *et al.*, "Peptide Synthesis", Second Edition, 1976, and K. D. Kopple, "Synthesis of Cyclic Peptides", *J. Pharm. Sci.*, 61 (9), 1345 (September 1972).

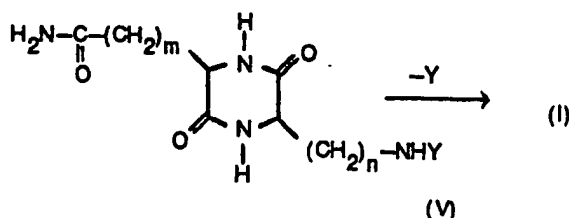
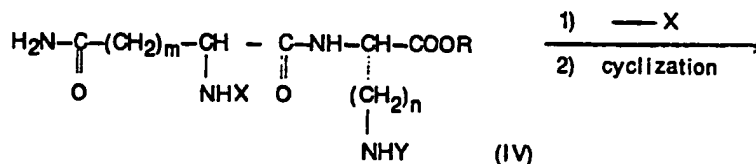
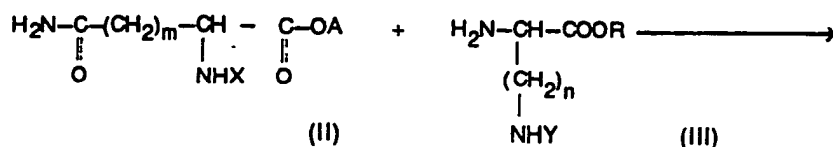
In this general scheme of preparation, the alpha-amino group of one of the constituent amino acids is protected on its alpha-amino group by allowing it to react with a reagent which will introduce a protecting group, as discussed further below. This protected constituent amino acid is then activated with respect to nucleophilic attack at its carboxy group by an amine, to form a carboxy activated protected constituent amino acid, as further described below. It is preferred that the constituent amino acid so treated is the glutamine or asparagine amino acid constituent.

The second constituent amino acid is then esterified on its carboxy terminus with, for example, a C₁-C₆ lower alcohol and (if appropriate) protected on its delta or epsilon amino group by allowing it to react with a reagent which will introduce a protecting group into that position. It is preferred that this amino acid constituent be lysine or ornithine.

Approximately molar equivalent amounts of each of these materials are allowed to react to form the resultant linear protected dipeptide, which is cyclized after removal of the alpha-amino-terminal protecting group. This cyclization may be accomplished by a variety of methods, including the active ester and azide methods and others well-known in the art. However, the direct method employing a carbodiimide (e.g., an N,N'-dialkylcarbodiimide, N,N'-dicyclohexylcarbodiimide, or N-ethyl-N'-(3-dimethylaminopropyl)carbodiimide, which is preferred, is illustrated below and employed in the Examples.

Finally, the delta or epsilon amino protecting group is removed, yielding the desired cyclic dipeptide as either a free base or acid addition salt.

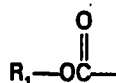
This reaction scheme may be illustrated by the following, wherein m and n are as previously defined, X and Y are amino protecting groups, R is loweralkyl, and A is a carboxy activating group:



The amino protecting groups X and Y may be the same or different and should be stable to removal by the steps employed to join the two amino acids, while X should be removable under conditions which will not remove Y, and Y should be removable under conditions which will not destroy the resulting diketopiperazine compound.

Exemplary of suitable amino-protecting groups are those of formula:

a)



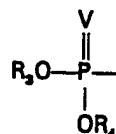
wherein R_1 is aryl (such as phenyl, tolyl, or xylyl); adamantyl; monosubstituted methyl (such as allyl, beta-cyanoethyl, fluorenylmethyl, benzyl, or benzyl wherein the phenyl ring is substituted with from one to three members selected from halo, nitro, C_1-C_6 loweralkyl, and C_1-C_6 loweralkoxy); disubstituted methyl (such as diisopropylmethyl, diphenylmethyl, cyclohexyl, cyclopentyl, or vinyl); or trisubstituted methyl (such as *t*-butyl, *t*-amyl, dimethyltrifluoromethylmethyl, or dimethylbiphenylmethyl);

b)



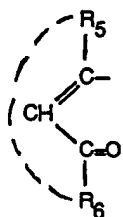
wherein R_2 is loweralkyl of two to four carbons such as ethyl, isopropyl, *t*-butyl, or loweralkyl of one to four carbons substituted with from one to five halo groups such as trifluoromethyl, chloromethyl, penta-chloroethyl;

c)

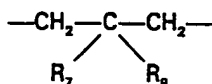


wherein V is S or O and R_3 and R_4 are each benzyl or C_1-C_6 loweralkyl);

d)

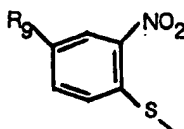


wherein R_5 and R_6 taken individually are each C_1-C_6 loweralkyl or R_5 and R_6 taken together is



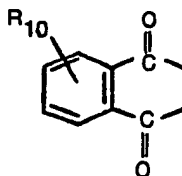
wherein R_7 and R_8 are each hydrogen or C_1-C_6 loweralkyl; and

e)



wherein R_9 is hydrogen or nitro;

f)



wherein R_{10} is hydrogen, methyl, halo, or nitro.

As used herein, "halo" includes fluoro, chloro, bromo, and iodo, but chloro and bromo are preferred. The terms "loweralkyl" and "loweralkoxy" include, respectively, saturated aliphatic hydrocarbons of one to six carbons such as methyl, ethyl, isopropyl, *t*-butyl, *n*-hexyl, and the corresponding alkoxies such as methoxy, ethoxy, isopropoxy, *t*-butoxy, *n*-hexoxy, and the like. Methyl is the preferred loweralkyl and methoxy is the preferred loweralkoxy.

The reagents employed to introduce these protecting groups (usually the corresponding acid chlorides, although other derivatives may be used) are sometimes referred to herein as "protecting group reagents". Other suitable protective groups are disclosed in, for example, "Protective Groups in Organic Chemistry", J. F. W. McOmie, ed., Plenum Press, N.Y., 1973.

It is preferred that X be *t*-butoxycarbonyl (BOC) and Y be benzyloxycarbonyl (CBZ).

A variety of reagents may be employed for producing the carboxy activated protected amino acid by introducing the A group.

One type of carboxy activated protected amino acid residue is a reactive ester. Exemplary of agents used to prepare the suitable active esters are phenol; phenol wherein the phenyl ring is substituted with one to five members selected from halo (e.g., chloro or fluoro), nitro, cyano, and methoxy; thiophenyl; N-hydroxyphthalimide; N-hydroxysuccinimide; N-hydroxyglutarimide; N-hydroxybenzamide; 1-hydroxybenzotriazole. Other suitable agents are disclosed in, for example, "Protective Groups in Organic Chemistry", J. F. W. McOmie, ed., referred to above. The specific examples provided below employ ortho-nitrophenol.

Other activation methods, such as the mixed or symmetrical anhydride method, the acid chloride method, and the azide method, are well-known in the art, being described in, e.g., Bodanszky *et al.*, "Peptide Synthesis", 2nd ed., 1976, pp 85-128. These other methods may also be employed.

The subject compounds exhibit immunoregulatory activity as demonstrated in an *in vitro* cytotoxic lymphocyte precursor unit (CLP-U) assay, in which the effect of *in vitro* incubation of one of

the subject compounds with mouse spleen cells on the generation of CLP—U was evaluated. This assay correlates directly with immune response in warm-blooded animals, including humans. That is, if a tested compound stimulates or inhibits the CLP—U generated in the assay at a certain concentration, these results indicate the immunoregulatory effect of the tested compound. This assay has also been
 5 conducted, e.g., for the known immunoregulatory peptide TP5, which has confirmed this correlation. For a more detailed description of this assay and its use for measuring immune regulation, reference is made to C. Y. Lau and G. Goldstein, *J. Immunology*: 124 (4), 1861—1865 (April 1980).

Because of these characteristics of the subject compounds, they are therapeutically useful in the treatment of humans and animals since they have the capability for correcting immune deficiencies in
 10 subjects in need of such correction. As a result, the products of this invention are considered to have multiple therapeutic uses. Because of their biological characteristics, which are extremely active at low concentrations, they are considered useful in assisting the collective immunity of the body in that the compounds will increase or assist in therapeutic stimulation of cellular immunity and thereby become useful in the treatment of diseases involving chronic infection *in vivo*, such as fungal or mycoplasma
 15 infections, tuberculosis, leprosy, and acute and chronic viral infections. Further, the compounds are considered to be useful in any area in which cellular immunity is an issue and particularly where there are deficiencies in immunity such as in the DiGeorge Syndrome (congenital absence of thymus). Also, where there is an excess of antibody production, the compounds can correct this condition by regulating T cell production. Thus, they may be of therapeutic use in certain autoimmune diseases in
 20 which damaging antibodies are present, for example, systemic lupus erythematosus and rheumatoid arthritis. Further, because of the characteristics of the compounds, they have *in vitro* usefulness in inducing the development of surface antigens of T cells, in inducing the development of the functional capacity to achieve responsiveness to mitogens and antigens and cell collaborativity in enhancing the ability of B cells to produce antibodies. Still further, the compounds are useful in treatment of
 25 conditions such as neoplasia in which the immune response is subnormal. Administration of an effective immunoregulatory amount of the subject compounds will assist in the treatment of such conditions (and especially neoplasia) either alone or in adjunct with other forms of treatment such as surgical removal.

The compounds of the present invention are therefore suitable for the regulation of the immune
 30 system of a subject, human or animal, in need of such immune regulation which comprises administration to said subject an effective immunoregulatory amount of one of the subject compounds, preferably in admixture with a pharmaceutical carrier. As used therein, the term "regulate" means that the subject compounds cause the immune system to return from an abnormal, diseased state to a normal, balanced state.

A further important property of the compounds of this invention is that they are highly active in
 35 very low concentrations ranging from 0.1 pg/ml. The carrier may be any of the well-known carriers for this purpose including normal saline solutions, preferably with a protein diluent such as bovine serum albumin (BSA) to prevent adsorptive losses to glassware at these low concentrations. The compounds of this invention are active parenterally at about 1 ng/kg of body weight. For the treatment of
 40 rheumatoid arthritis, they may generally be administered at a rate of about 0.1 to 100 ng/kg of body weight. Generally, the same range of dosage amounts may be used in treatment of the other conditions or diseases mentioned.

To prepare the pharmaceutical compositions of the present invention, a compound of Formula (I) or an acid addition salt thereof is combined as the active ingredient in intimate admixture with a
 45 pharmaceutical carrier according to conventional pharmaceutical compounding techniques, which carrier may take a wide variety of forms depending on the form of preparation desired for administration, e.g., sublingual, rectal, nasal, oral, or parenteral. In preparing the compositions in oral dosage form, any of the usual pharmaceutical media may be employed, such as for example, water, glycols, oils, alcohols, flavoring agents, preservatives or coloring agents in the case of oral liquid preparations
 50 such as for example, suspensions, elixirs, and solutions; or carriers such as starches, sugars, diluents, granulating agents, lubricants, binders or disintegrating agents in a case of oral solid preparations, such as for example, powders, capsules, and tablets. Because of their ease in administration, tablets and capsules represent the most advantageous oral dosage unit form, in which case solid pharmaceutical carriers are obviously employed. If desired, tablets may be sugar coated or enteric coated by standard
 55 techniques. For parenterals, the carrier will usually comprise sterile water, although other ingredients, for example, to aid solubility or for preservative purposes, may be included. Injectable suspensions may also be prepared, in which case appropriate liquid carriers, suspending agents, and the like may be employed. The parenteral pharmaceutical compositions of the invention should be designed to administer the subject compounds at a rate of about 0.1 to about 100 ng/kg of body weight. The oral
 60 compositions should administer about 100 to 1000 times the dose for parenteral administration — i.e., from about 10 ng/kg to about 100 μ g/kg of body weight. Accordingly, the parenteral compositions should contain, per dosage unit, from about 5 ng to about 5 μ g, whereas the oral compositions should contain, per dosage unit, from about 500 ng to about 5 mg of the subject compound.

The preparation and evaluation of an exemplary compound and the invention is given below for
 65 purposes of illustration. Unless otherwise stated, all parts are by weight.

Example I

A. *N*^ε-BOC-L-Glutaminyl-*N*^ε-Benzyloxycarbonyl-L-Lysine-OMe

BOC-Gln-ONp (3.67 g; 0.01 moles) and *N*^ε-Z-Lys-OMe.HCl (3.64 g; 0.011 moles) were dissolved in 30 ml of DMF. The mixture was cooled in an ice bath and diisopropylethylamine (1.91 ml; 0.011 moles) was added slowly with stirring. The reaction was stirred for 3 days at ambient temperature. The solvent was removed under reduced pressure and the residue was redissolved in ethylacetate (200 ml) and washed with 10% sodium bicarbonate solution (6 x 50 ml), H₂O (3 x 50 ml), 0.1 N HCl (3 x 50 ml) and H₂O (3 x 50 ml). The organic layer was dried over sodium sulfate and after filtration, the solvent was removed under reduced pressure. The crude product (4.6 g) was obtained by adding ether to the mixture, removing the solid by filtration, washing with ether and drying *in vacuo*. This material was suspended in ethylacetate, filtered and washed with minimum amount of ethylacetate. The product was dried *in vacuo* to give a white solid (3.8 g, 73% of yield), m.p. 99–100°C; Rf 0.49 (CHCl₃/MeOH/HOAc = 85:10:5; Silica Gel GF 250 microns); pmr (CD₃OD)δ, 1.42 [s, 9H, (CH₃)₃C-], 1.5–2.4 (m, 10H, methylene H), 3.1 (t, 2H, CH₂-NH-Z), 3.7 (s, 3H, -OCH₃), 4–4.5 (m, 2H, α-CH-), 5.1 (s, 2H, -CH₂-C₆H₅), 7.39 (s, 5H, -C₆H₅).

Anal. Calcd. for C₂₅H₃₈N₄O₈: C, 57.46; H, 7.33; N, 10.71

Found: C, 57.23 H, 7.24; N, 10.52

B. *cyclo*-(L-Glutaminyl-*N*^ε-Benzyloxycarbonyl-L-lysine)

BOC-Gln-*N*^ε-CBZ-Lys-OMe (2.15 g; 4.11 mmoles) was treated with 10 ml of 4N HCl/Dioxane for 1h at room temperature. The solvent was removed under reduced pressure and the residue was dissolved in 25 ml of CH₂Cl₂. The solution was cooled down to 0°C and diisopropylethylamine (0.96 ml; 4.11 mmoles) was added slowly under stirring. The mixture was taken down to dryness and the remaining material was dissolved in 50 ml of CH₃OH and refluxed gently for 24h. The mixture was placed in the cold room and the precipitate was collected by filtration and washed with water thoroughly. The product was dried *in vacuo* to give a white solid, (300 mg; 24% of yield); m.p. 235°C; a second crop of material was obtained (400 mg; 26% of yield); Rf 0.256 (n-Butanol/H₂O/HOAc = 3:1:1, Silica Gel GF, 250 microns); pmr (TFA)δ, 1.4–2.8 (m, 12H, methylene H), 4.2–4.5 (m, 2H, α-CH), 5.2 (s, 2H, -CH₂-C₆H₅); 7.32 (s, 5H, -C₆H₅).

Anal. Calcd. for C₁₉H₂₆N₄O₆: C, 58.45; H, 6.71; N, 14.34

Found: C, 58.31; H, 6.61; N, 14.30

C. *cyclo*-(L-Glutaminyl-L-lysine).Hydrochloride

cyclo-(L-Gln-*N*^ε-Benzyloxycarbonyl-L-Lys) (140 mg; 0.48 mmoles) was suspended in 10 ml. of acetic acid and 10 ml of 1N HCl. To the suspension, 100 mg of 10% Pd/C was added slowly. The mixture was hydrogenated on a Parr-shaker at 40 psi for 24h. The catalyst was removed by filtration and the filtrate was taken down to dryness. To the residue was added 15 ml of ethanol and few drops of water. The mixture was placed in the cold room overnight and the precipitate was collected by filtration and dried *in vacuo*. The yield was 95% (57 mg, 1st crop and 40 mg, 2nd crop); m.p. 246–248°C. Rf 0.20 (n-Butanol/HOAc/H₂O = 3:1:1, Silical Gel GF, 250 microns); M.S. (probe): m/e 256 (M⁺); pmr (D₂O)δ 1.3–2.4 (m, 10H, methylene H), 2.8–3.1 (m, 2H, -CH₂-NH 4.05 (t, 2H, α-CH-).

Anal. Calcd. for C₁₁H₂₀N₄O₃: C, 45.13; H, 7.23; N, 19.13

Found: C, 45.42; H, 7.10; N, 18.41

Test Results

To evaluate the immunoregulatory activity of the cyclic dipeptide produced in Example III, the following assay was conducted.

Single cell suspensions were made from the spleens of normal C57BL/6 mice (female, 8 weeks old, Jackson Laboratory, Bar Harbour, Maine) and the cells were washed three times with PBS. The cells were then resuspended in RPMI 1640 medium (Gibco) and then incubated with various concentrations of the test peptide for 1h at 37°C. After incubation, the cells were washed and the frequency of CLP-U was estimated by the limiting dilution assay according to the method of Teh, *et al.*, J. Immunol. 118: 1049 (1977) and Lindahl and Wilson, J. Exp. Med. 145:508 (1977).

Limiting numbers of C57BL/6 spleen cells (2 x 10⁴ to 8 x 10⁴) were put in V-bottom Linbro trays (Flo Laboratories) together with mitomycin-C-treated DBA spleen cells (DBA/2J mice, Jackson Laboratory, Bar Harbour, Maine), 10⁷ DBA cells having been treated with 30 μg of mitomycin-C for 30 minutes at 37°C. The number of DBA cells used varied from 1.2 x 10⁵ to 6.5 x 10⁵ per culture. Twenty replicates were set up for each cell concentration.

The combined cultures were then incubated at 37°C for six days, at the end of which period 100 μ l of the supernatant medium was removed from each well, and the cells were then mixed with 0.1 ml of fresh medium containing 2×10^4 P815 mastocytoma cells labeled with ^{51}Cr as described previously by Teh, *et al.* After a further incubation period of 4h, 100 μ l of the supernatant fluid in each well was removed and the radioactivity therein was determined in a gamma spectrometer. The background chromium release from cultures containing only C57BL/6 cells or only DBA cells was always less than 15%.

The frequencies of CLP—U were calculated according to Poisson's statistics as follows. First, the mean spontaneous release was calculated by averaging the counts of twenty wells that received only C57BL/6 cells. Wells were scored as positive if their counts were greater than 2.07 standard deviations above this mean spontaneous release value ($P < 0.05$). According to Poisson's distribution, the frequencies of precursors can then be calculated by the equation $P = E^{\delta N}$ where P equals the probability of response, δ equals frequency, and N equals the number of cells put in the culture.

The percent of non-responding cultures for each C57BL/6 concentration was calculated and plotted on semi-logarithmic scale as a function of the cell concentration. According to the equation given above, the inverse of the number of cells in culture that will give 37% non-responding culture is the frequency of precursors in that culture. The best fit regression line by least square method was calculated for each line and the frequency was then estimated.

Enhancement of CLP—U upon suboptimal stimulation was observed for the cyclic dipeptide of Example III at concentrations ranging from 0.1 μ g/ml through 10 ng/ml, although optimal enhancement was observed from 0.1 μ g/ml through 0.01 ng/ml.

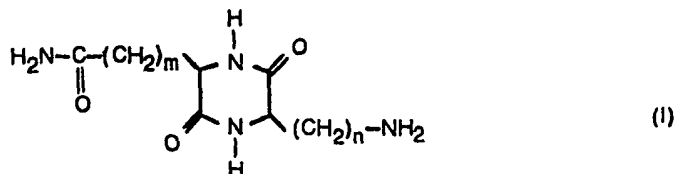
Example II

Following the procedures of Examples I—III, but substituting for the reagents used therein equivalent amounts of suitable starting materials, there are prepared the following: cyclo-(GLN—LYS), cyclo-(GLN—ORN), cyclo-(ASN—LYS), cyclo-(ASN—ORN), cyclo-(D—GLN—D—LYS), cyclo-(D—GLN—D—ORN), cyclo-(D—ASN—D—LYS), cyclo-(D—ASN—D—ORN), cyclo-(D—GLN—LYS), cyclo-(GLN—D—LYS), cyclo-(ASN—D—LYS), cyclo-(GLN—D—ORN), and cyclo-(D—ASN—ORN).

These compounds also possess immunoregulatory activity as demonstrated by the test described above.

Claims

1. A chemical compound of formula:



and optical isomers and pharmaceutically acceptable acid addition salts thereof, wherein m is 1 or 2 and n is 3 or 4.

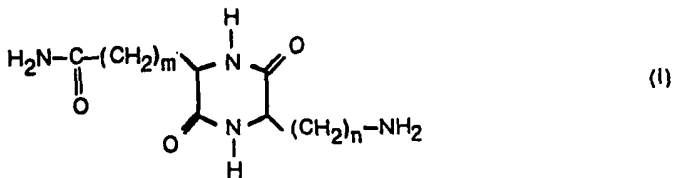
2. A chemical compound selected from the group consisting of cyclo-(GLN—LYS), cyclo-(GLN—ORN), cyclo-(ASN—LYS), cyclo-(ASN—ORN), and pharmaceutically acceptable acid addition salts thereof.

3. A chemical compound selected from the group consisting of cyclo-(D—GLN—D—LYS), cyclo-(D—GLN—D—ORN), cyclo-(D—ASN—D—LYS), cyclo-(D—ASN—D—ORN), and the pharmaceutically acceptable acid addition salts thereof.

4. The chemical compound cyclo-(GLN—LYS) and pharmaceutically acceptable acid addition salts thereof.

5. An immunoregulatory pharmaceutical composition comprising, in admixture with a pharmaceutically acceptable vehicle, an effective immunoregulatory amount of the compound of any one of Claims 1 to 4.

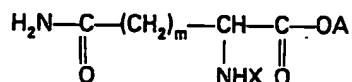
6. A method of preparing a chemical compound of formula:



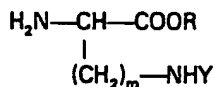
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wherein m is 1 or 2 and n is 3 or 4 which comprises:

a) reacting approximately m lar equivalents of a comp und of f rmula:

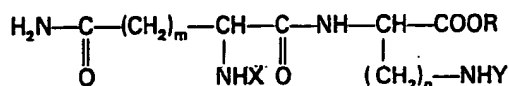


and a compound of formula:



(III)

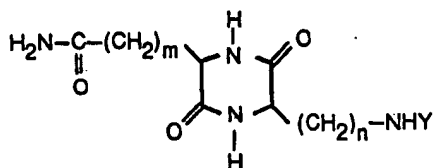
to form a compound of formula:



(IV)

b) removing the X protecting group from compound (IV)

c) cyclizing the resultant compound to form a compound of formula:



(IV);

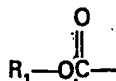
and

d) removing the Y protecting group from compound (V), whereby compound (I) is formed;

wherein:

X and Y are each selected from the group consisting of:

a)



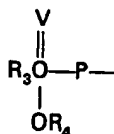
wherein R₁ is phenyl; tolyl; xylol; adamantyl; allyl; beta-cyanoethyl; fluorenylmethyl; benzyl, benzyl wherein the phenyl ring is substituted with from one to three members selected from halo, nitro, C₁-C₆ loweralkyl, and C₁-C₆ loweralkoxy, diisopropylmethyl; diphenylmethyl; cyclohexyl; cyclopentyl; vinyl; t-butyl; t-amyl; dimethyltrifluoromethylmethyl; or dimethyl-biphenylmethyl;

b)



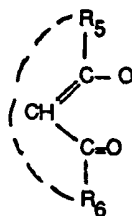
wherein R₂ is loweralkyl of two to four carbons or loweralkyl of one to four carbons substituted with from one to five halo groups;

c)

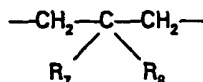


wherein V is S or O and R₃ and R₄ are each benzyl or C₁-C₆ loweralkyl;

d)

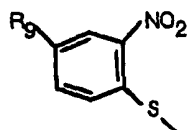


wherein R_5 and R_6 taken individually are each C_1-C_6 loweralkyl or R_5 and R_6 taken together is



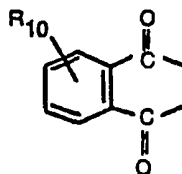
R_8 are each hydrogen or C_1-C_6 loweralkyl;

e)



wherein R_9 is hydrogen or nitro; and

f)



wherein R_{10} is hydrogen, methyl, halo, or nitro; and

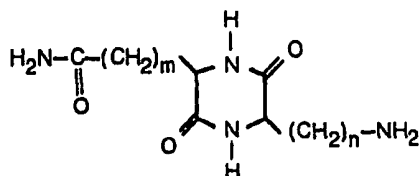
A is benzyl or benzyl in which the phenyl group is substituted with from one to three members each selected from halo, nitro, C_1-C_3 loweralkyl, and C_1-C_3 loweralkoxy.

7. A pharmaceutical composition according to Claim 5 in unit dosage form, comprising, in the case of a parenteral composition from about 5 ng to about 5 μ g of said compound, or, in the case of an oral composition, from about 500 ng to about 5 mg of said compound.

8. A compound in accordance with any one of Claims 1 to 4, or a composition in accordance with Claim 5 or Claim 7, for use as an immunoregulatory agent.

Revendications

1. Composé chimique de formule



(I)

et ses isomères optiques et sels d'addition d'acides pharmaceutiquement acceptables, où n vaut 1 ou 2, et n vaut 3 ou 4.

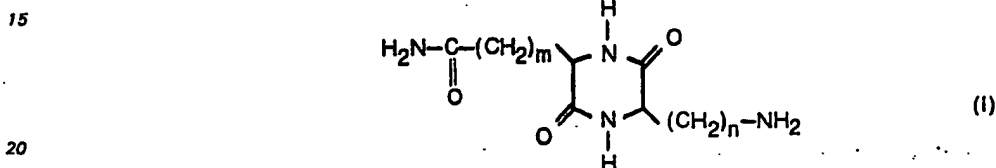
2. Comp sé chimique choisi dans le groupe onstitué par cyclo-(GLN—LYS), cyclo-(GLN—ORN), cyclo-(ASN—LYS), cyclo-(ASN—ORN), et leurs sels d'addition d'acides pharmaceutiquement acceptabl s.

3. Composé chimique choisi dans le groupe constitué par cyclo-(D-GLN-D-LYS), cyclo-(D-GLN-D-ORN), cyclo-(D-ASN-D-LYS), cyclo-(D-ASN-D-ORN), et leurs sels d'addition d'acides pharmaceutiquement acceptables.

4. Composé chimique cyclo-(GLN—LYS) et ses sels d'addition d'acides pharmaceutiquement acceptables.

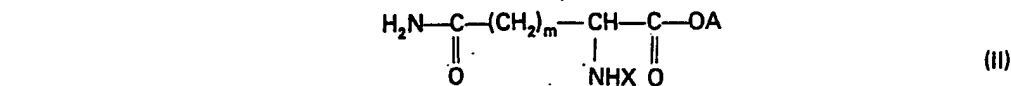
10 5. Composition pharmaceutique immuno-régulante comprenant, mélangée à un véhicule pharmaceutiquement acceptable, une quantité immuno-régulante efficace du composé de l'une quelconque des revendications 1 à 4.

6. Procédé de préparation d'un composé chimique de formule

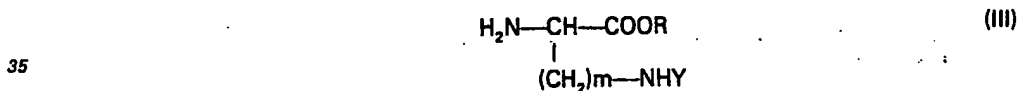


Où m vaut 1 ou 2 et n vaut 3 ou 4 dans lequel on procède:

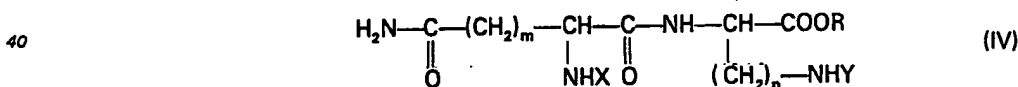
a) en faisant réagir des équivalents molaires d'un composé de formule



30 et d'un composé de formule

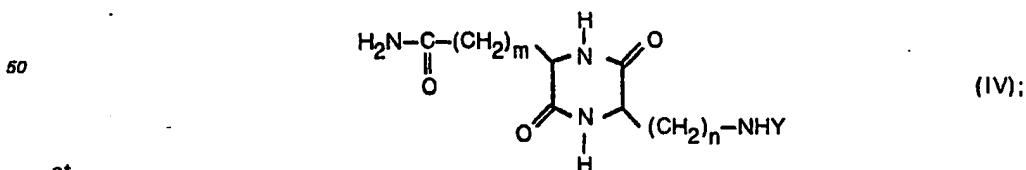


pour former un composé de formule



b) en enlevant le groupe protecteur X du composé (IV)

45 c) en cyclisant le composé obtenu pour former un composé de formule



et

55 d) en enlevant le groupe protecteur Y du composé (V), grâce à quoi on forme le composé (I);

où

X et Y sont l'un et l'autre choisis dans le groupe constitué par:

a)



65 où R₁ est un phényle, un tolyle, un xyle, un adamantyle, un allyle, un bêta-cyanoéthyle, un fluorényl-méthyle, un benzyle, un benzyle où le noyau phényle est substitué par 1 à 3 éléments

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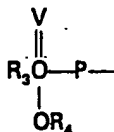
choisis entre halo, nitro, alcoyle inférieur en C₁ à C₆ et alcoxy inférieur en C₁ à C₆, diisopropyl-méthyle, diphenylméthyle, cyclohexyle, cyclopentyle, vinyle, t-butyle, t-amyle, diméthyltri-fluorométhylméthyle, ou diméthylbiphenylméthyle;

b)



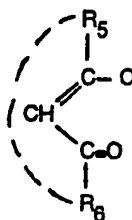
où R₂ est un alcoyle inférieur en C₂ à C₄ ou un alcoyle inférieur en C₂ à C₄ substitué avec de 1 à 5 groupes halo;

c)

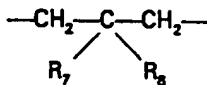


où V représente S ou O et R₃ et R₄ représentent chacun un benzyle ou un alcoyle inférieur en C₁ à C₆;

d)

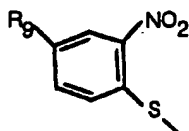


où R₅ et R₆ pris isolément représentent chacun un alcoyle inférieur en C₁ à C₆ ou bien où R₅ et R₆ pris ensemble représentent



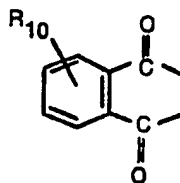
Où R₇ et R₈ représentent chacun un hydrogène ou un alcoyle inférieur en C₁ à C₆;

e)



où R₉ est un hydrogène ou un nitro; et

f)



où R₁₀ est un hydrogène, un méthyle, un halo ou un nitro; et A est un benzyle ou un benzyle où le groupe phényle est substitué avec 1 à 3 éléments choisi(s) chacun entre halo, nitro, alcoyle inférieur en C₁ à C₃, t alcoxy inférieur en C₁ à C₃.

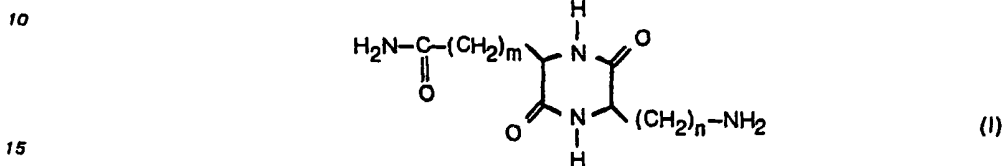
7. Comp sition pharmaceutique sel n la revendicati n 5 s us f rme p sol gique unitaire com-
prenant, dans le cas d'une composition parentérale, d' nviron 5 ng à enviro n 5 µg dudit c mposé u,

dans le cas d'une composition orale, d'environ 500 ng à environ 5 mg dudit composé.

8. Composé selon l'une quelconque des revendications 1 à 4, ou composition selon la revendication 5 ou la revendication 7, aux fins d'application comme agent immuno-régulant.

5 Patentansprüche

1. Eine chemische Verbindung mit der Formel:



und die optischen Isomeren und pharmazeutisch annehmbaren Säureadditionssalze hiervon, worin m 1 oder 2 und n 3 oder 4 bedeutet.

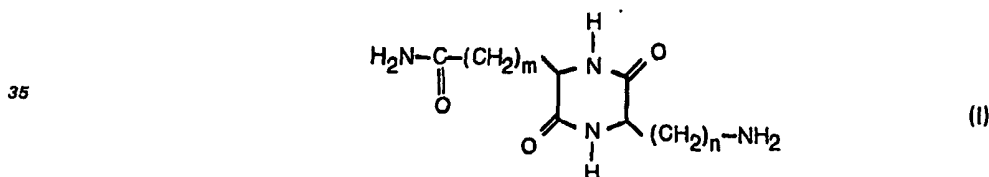
2. Eine chemische Verbindung, ausgewählt aus der cyclo-(GLN-LYS), cyclo-(GLN-ORN), cyclo-(ASN-LYS), cyclo-(ASN-ORN) und die pharmazeutisch annehmbaren Säureadditionssalze hiervon umfassenden Gruppe.

3. Eine chemische Verbindung, ausgewählt aus der cyclo-(D-GLN-D-LYS), cyclo-(D-GLN-D-ORN), cyclo-(D-ASN-D-LYS), cyclo-(D-ASN-D-ORN) und die pharmazeutisch annehmbaren Säureadditionssalze hiervon umfassenden Gruppe.

4. Die chemische Verbindung cyclo-(GLN-LYS) und die pharmazeutisch annehmbaren Säureadditionssalze hiervon.

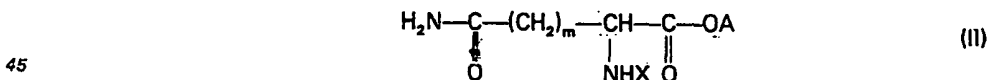
5. Eine immunregulierende pharmazeutische Zusammensetzung, enthaltend im Gemisch mit einem pharmazeutisch annehmbaren Träger eine wirksame immunregulierende Menge der Verbindung nach einem der Ansprüche 1 bis 4.

6. Ein Verfahren zur Herstellung einer chemischen Verbindung mit der Formel:

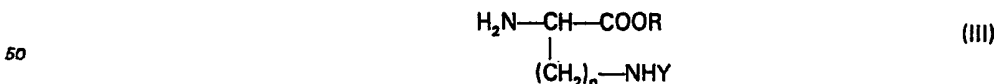


worin m 1 oder 2 und n 3 oder 4 bedeuten, umfassend:

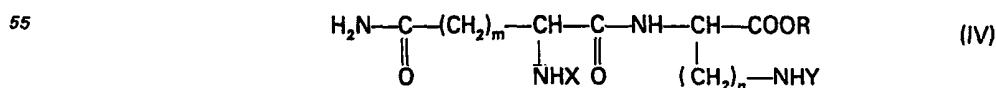
a) Umsetzen von ungefähr molaren Äquivalenten einer Verbindung der Formel:



und einer Verbindung der Formel:

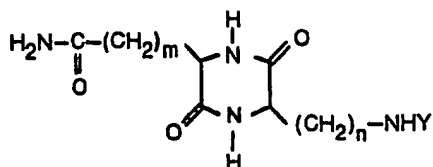


unter Ausbildung einer Verbindung der Formel:



b) Abtrennen der Schutzgruppe X aus der Verbindung (IV)

c) Cyclisieren der erhaltenen Verbindung unter Ausbildung einer Verbindung der Formel:



(IV);

(V) und

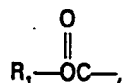
10 d) Abtrennen der Schutzgruppe Y aus der Verbindung (V), wodurch die Verbindung (I) gebildet wird;

wobei:

X und Y jeweils ausgewählt sind aus der Gruppe bestehend aus:

a)

15



20 worin R_1 für Phenyl; Töyl; Xyl; Adamantyl; Allyl; beta-Cyanoethyl; Fluorenylmethyl; Benzyl; im Phenylring durch 1 bis 3 Reste, ausgewählt unter Halogen, Nitro, C_1 — C_6 -Niederalkyl und C_1 — C_6 -Niederalkoxy, substituiertes Benzyl; Diisopropylmethyl; Diphenylmethyl; Cyclohexyl, Cyclopentyl; Vinyl; tert. Butyl; tert. Amyl; Dimethyltrifluormethylmethyl; oder Dimethylbiphenylmethyl steht;

b)

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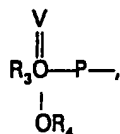


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worin R_2 Niederalkyl mit 2 bis 4 Kohlenstoffatomen oder durch eine bis zu fünf Halogensubstituenten substituiertes Niederalkyl mit 1 bis 4 Kohlenstoffatomen darstellt;

c)

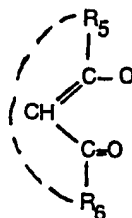
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d) worin V für S oder O steht und R_3 und R_4 jeweils Benzyl oder C_1 — C_6 -Niederalkyl bedeuten;

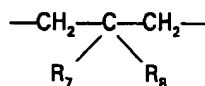
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worin R_5 und R_6 jedes für sich allein jeweils C_1 — C_6 -Niederalkyl bedeuten oder R_5 und R_6 zusammen für

55



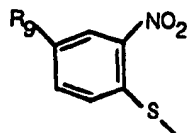
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stehen, worin R_7 und R_8 jeweils Wasserstoff oder C_1 — C_6 -Niederalkyl darstellen;

65

e)

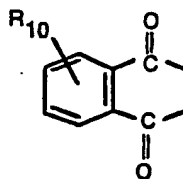
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f) worin R_9 Wasserstoff oder Nitro bedeutet; und

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worin R_{10} für Wasserstoff, Methyl, Halogen oder Nitro steht; und
A Benzyl oder in der Phenylgruppe durch 1 bis 3 Reste, jeweils ausgewählt unter Halogen, Nitro,
 C_1-C_3 -Niederalkyl und C_1-C_3 -Niederalkoxy, substituiertes Benzyl bedeutet.

25 7. Eine pharmazeutische Zusammensetzung nach Anspruch 5 in Dosisform, enthaltend im
Falle einer parenteralen Zusammensetzung von etwa 5 ng bis etwa 5 μ g der genannten Verbindung,
oder, im Falle einer oralen Zusammensetzung, von etwa 500 ng bis etwa 5 mg der genannten Ver-
bindung.

8. Eine Verbindung gemäß einem der Ansprüche 1 bis 4, oder eine Zusammensetzung gemäß An-
spruch 5 oder Anspruch 7, zur Anwendung als ein Immunregulierendes Mittel.

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